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Published in:
FEBS Letters

DOI:
[10.1016/j.febslet.2007.04.015](https://doi.org/10.1016/j.febslet.2007.04.015)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2007

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Tomkiewicz, D., Nouwen, N., & Driessen, A. J. M. (2007). Pushing, pulling and trapping - Modes of motor protein supported protein translocation. *FEBS Letters*, 581(15), 2820-2828.
<https://doi.org/10.1016/j.febslet.2007.04.015>

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Minireview

Pushing, pulling and trapping – Modes of motor protein supported protein translocation

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Received 26 February 2007; revised 1 April 2007; accepted 11 April 2007

Available online 18 April 2007

Edited by Thomas C. Marlovits

Abstract Protein translocation across the cellular membranes is an ubiquitous and crucial activity of cells. This process is mediated by translocases that consist of a protein conducting channel and an associated *motor protein*. Motor proteins interact with protein substrates and utilize the free energy of ATP binding and hydrolysis for protein unfolding, translocation and unbinding. Since motor proteins are found either at the *cis*- or *trans*-side of the membrane, different mechanisms for translocation have been proposed. In the Power stroke model, *cis*-acting motors are thought to push, while *trans*-motors pull on the substrate protein during translocation. In the Brownian ratchet model, translocation occurs by diffusion of the unfolded polypeptide through the translocation pore while directionality is achieved by trapping and refolding. Recent insights in the structure and function of the molecular motors suggest that different mechanisms can be employed simultaneously.

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Keywords: Translocation; Molecular motor; Secretory protein; SecA; BiP; Hsp70

1. Introduction

More than 30% of the proteins synthesized in the cytosol function in an organelle or outside the cell. Consequently, these proteins have to cross at least one lipid membrane to reach their final destination. As membranes act as hydrophobic barriers that are intrinsically impermeable for ions and polar solutes, the question arises: “How does membrane passage of proteins occur?” Essentially, protein translocation is an energy requiring and protein-mediated process. Protein translocation systems present in different membranes and organelles have several fea-

tures in common. They comprise a protein conducting channel (PCC) and a motor protein. The pore constitutes a hydrophilic interior that allows membrane passage of proteins, usually in an unfolded state. During post-translational translocation, proteins are first synthesized to their full length at the ribosome as a precursor with an N-terminal signal sequence (preprotein) whereupon they are translocated across the membrane by the action of *cis*- or *trans*-acting motors (Fig. 1A). These *molecular motors* usually are ATPases that can bind preproteins reversibly and drive their translocation either by pulling, pushing or trapping mechanisms [2] (Table 1). Protein translocation can also be coupled directly to polypeptide chain elongation at the ribosome, a process termed co-translational translocation that will not be further discussed here.

Currently, there are two major mechanistic models that describe the role of motor proteins in post-translational preprotein translocation: the *power-stroke* model and the *Brownian ratchet* (Fig. 1B). In the first model, ATP binding and hydrolysis lead to conformational changes in the motor protein, which translate into a mechanical force that is imposed on the associated preprotein substrate. This results in movement of the preprotein across the membrane. With *cis*-acting motors, the force imposed reflects a pushing movement, while *trans*-acting motors generate a pulling force on the protein [2]. With the Brownian ratchet, the motor protein is a device which biases the random Brownian motion of an unfolded polypeptide chain [69]. The spontaneous reversible movement (hysteresis) of the polypeptide in the translocation pore is coupled to an energy-requiring trapping by the motor protein. Trapping events prevents retrograde movement, thereby giving directionality to the translocation process [51,2].

In addition to ATP binding and hydrolysis, two alternative energy sources can be involved in protein translocation. Firstly, the entropic energy of protein folding and unfolding which is of primary importance for the Brownian motions of polypeptides [69]. Secondly, the proton motive force (PMF), which is composed of the transmembrane pH gradient (ΔpH) and electrical potential ($\Delta\psi$). In bacteria, both the ΔpH and $\Delta\psi$ can efficiently drive protein translocation in the absence of the motor protein once translocation has been initiated at the expense of ATP [64,14]. The exact mechanism of PMF-driven translocation is largely unknown. For the initiation of mitochondrial protein import, the $\Delta\psi$ is required [60].

Here, we will discuss the function of motor proteins in post-translational protein translocation, with an emphasis on protein folding and the translocation mechanism.

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Abbreviations: CTD, small C-terminal zinc-binding domain; ER, endoplasmic reticulum; JDP, J-domain protein; NBD, nucleotide binding domain; NEF, nucleotide exchange factor; PAM, presequence translocase-associated motor; PBD, preprotein binding domain; PCC, protein conducting channel; PMF, proton motive force; ΔpH , transmembrane pH gradient; $\Delta\psi$, transmembrane electrical potential

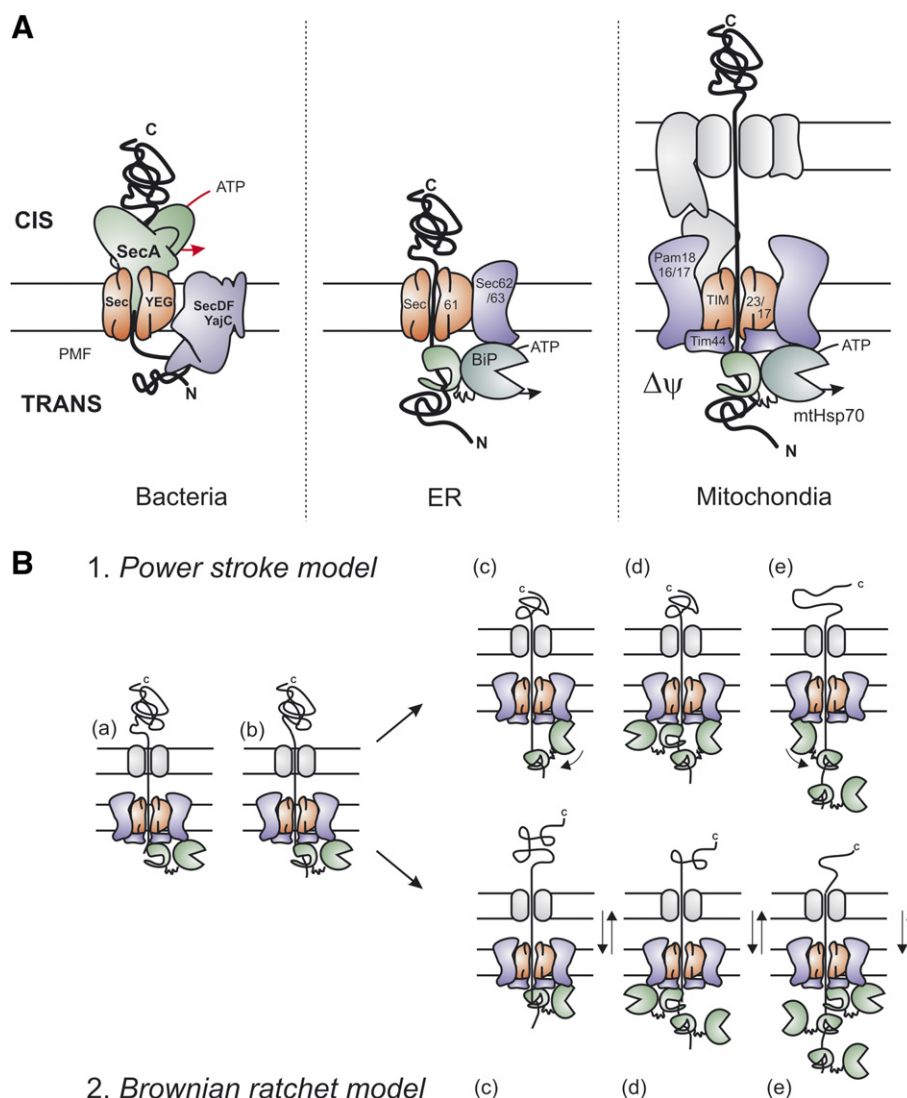


Fig. 1. Molecular motors. (A) Protein translocation systems in bacteria (*Escherichia coli*, SecYEG/SecA), the endoplasmic reticulum (ER, Sec61 $\alpha\beta\gamma$ /BiP) and mitochondria (TOM-TIM/mtHsp70). Translocation channels are in red, motor proteins in green, accessory proteins in blue. (B) Mechanistic models for the *trans*-motor Hsp70-dependent protein import into mitochondria. (1) Power stroke model: (a) The signal sequence is translocated to the *trans* side of the membrane in response to the $\Delta\psi$. ATP-bound luminal Hsp70 with an open peptide binding pocket interacts with the luminal domain of the J-domain protein (JDP) near the pore exit; (b) were it binds the emerging polypeptide; (c) Hsp70 undergoes a conformational change (arrow) upon polypeptide and JDP stimulated hydrolysis of ATP which result in a tight binding of the polypeptide and a perpendicular movement of the luminal Hsp70. The generated force unfolds the preprotein at the *cis* side and pulls the polypeptide to the *trans*-side of the membrane; (d) a second luminal Hsp70 binds to the polypeptide, hydrolyses ATP and (e) pulls the next segment of the preprotein into the matrix. (2) The Brownian ratchet model: (a) and (b) as above; (c) Brownian oscillations result in the forward and backward movements of unfolded polypeptide segments in the translocation channel. Upon ATP hydrolysis, the peptide binding pocket of Hsp70 closes around the incoming polypeptide and prevents backsliding and refolding of the preprotein at the *cis*-side; (d) Luminal Hsp70 dissociates from the JDP and after a sufficient length of polypeptide is translocated, a second luminal Hsp70 traps the polypeptide at the *trans*-side of the membrane; (e) the polypeptide slides back and forth in the import channel allowing consecutive cycles of Hsp70 trapping of the polypeptide at the pore exit site.

Table 1
Post-translational protein translocation systems involving motor proteins

	Motor protein	Energy	Membrane side	PCC	Additional proteins
Bacteria	SecA	ATP, PMF	<i>cis</i>	SecYEG	SecDF(yajC)
ER	BiP	ATP	<i>trans</i>	Sec61 $\alpha\beta\gamma$	Sec62/63, BAP, GRP170
Mitochondria	mtHsp70	ATP, $\Delta\psi$	<i>trans</i>	TIM23/17	Tim44, Pam18/16/17, Mge1

2. Protein folding and unfolding during translocation

Except for the twin arginine translocation system (for review see [36]), most translocases facilitate transmembrane move-

ment of unfolded polypeptides. This is consistent with the dimensions of the pores that can accommodate only unfolded polypeptide chains [62,65]. Therefore, the pore presents a major entropic barrier that hinders spontaneous movement of

polypeptides across the membrane. Protein folding on the *cis*-side of the membrane is a main factor that can interfere with translocation, while protein folding at the *trans*-side may promote translocation. Therefore, preproteins need either to be presented to the translocation system in an unfolded state or be unfolded actively. In bacteria, both the signal sequence and the molecular chaperone SecB contribute to stabilizing the unfolded state of the protein [61,35]. This unfolded state, also known as translocation competent state, is characterized by native-like secondary structure and undefined tertiary structure. In contrast, proteins presented in a folded state can still be imported into mitochondria, while the signal sequence in mitochondrial preproteins does not appear to alter the folding characteristics [83,25].

During translocation, preproteins may need to be further unfolded. In the Brownian ratchet model, proteins are stabilized in a loosely folded state by chaperones, spontaneously further unfold during translocation, and refold at the *trans*-side. The latter may be facilitated by post-translocation events such as disulfide bond formation, *cis*–*trans* prolyl peptide bond isomerisation and glycosylation. In the Power stroke model, proteins are actively unfolded during the translocation process. A detailed insight in the protein folding pathways and their energy characteristics could thus assist in distinguishing between these two models of translocation.

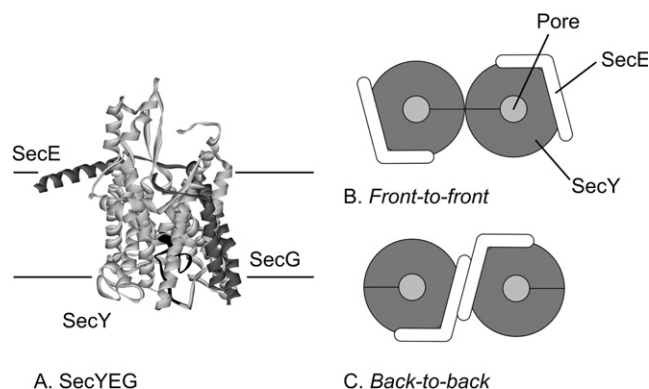
3. Sec-translocase – similar channel but different molecular motors

One of the best studied translocation systems is the Sec translocase, which enables protein translocation across the cytoplasmic membrane of prokaryotes, and the endoplasmic reticulum (ER) and thylakoid membranes in eukaryotes (for review see [56]). The PCC of the Sec translocase is highly conserved throughout all kingdoms of life (See Box 1), while the motor proteins differ in structure, mechanism and localization. In bacteria, the motor protein SecA drives translocation from the *cis*-side of the membrane, whereas protein import into the ER is actuated by the *trans*-acting BiP protein (Fig. 1).

4. *Cis*-acting motor protein – SecA

A possible mechanism for protein translocation was described on the basis of biochemical studies [64,77]. Herein, SecA uses the energy of ATP binding and hydrolysis to translocate preproteins in consecutive steps of about 40 amino acids per ATP cycle (for review see [8]). A single catalytic cycle consists of two distinct sub-steps; one driven by binding of SecA to the translocating preprotein and the second by binding of ATP to SecA [77]. The translocation time increases linearly with the length of the preprotein [74]. In this *step-wise mechanism*, SecA may act as Power stroke device, pushing preprotein segments with distinct size through the PCC.

Initially the *step-wise* translocation mechanism was linked to nucleotide-dependent conformational changes in SecA that would drive the partial co-insertion of SecA domains with the associated preprotein into the PCC [16]. However, the dimensions and the shape of the SecY channel seem too narrow to accommodate large SecA protein domains [75]. Evi-



Box 1. The Sec61/SecY channel. (A) The PCC is built from the Sec61 α /SecY (light grey) channel and the peripheral Sec61 γ /SecE (dark grey) and Sec61 β /SecG proteins (dark grey) (yeast/bacterial nomenclature). The channel forms an hourglass-like structure with a pore ring of hydrophobic residues (mainly isoleucines) at its constriction [75]. The pore is closed at the *trans*-side by a plug (black) formed by a short α -helix that folds back into the funnel. The clamshell-like structure of SecY comprises a lateral opening to the lipid bilayer which may function as exit site for hydrophobic transmembrane segments of translocating polypeptides during membrane insertion. Only the monomeric structure is shown. Biochemical data indicate that the active channel may consist of an oligomeric arrangement of SecYEG, possibly a dimer. In a proposed ‘front-to-front’ dimer (B), the two lateral gates of SecY face each other providing the possibility of a larger consolidated central channel [49]. In the alternative arrangement, the ‘back-to-back’ dimer (C), two SecYEG complexes interact through the highly tilted transmembrane domain of SecE (Sec61 γ) at the back of each of the monomeric channels [4]. This leaves the lateral gates exposed to the lipid bilayer, and constitutes a structure with two individual pores.

dence for membrane insertion was based mainly on the observation that most of the SecA structure (N- and C-terminal 60 and 30 kDa regions) adopts a protease-resistant conformation during translocation that can be reversed by detergent disruption of the membrane. However, the latter is due to the detergent susceptibility of SecA and of the SecA–SecY interaction [76]. Currently, the observed protease-resistant fragments are believed to represent translocation-relevant conformational states of SecA.

The elucidation of the structure of SecA and of the SecYEG complex, either alone or in association with the ribosome, now provides some new insights in the possible mode of action of SecA. SecA is a 100 kDa protein with five distinct domains (Fig. 2A) [26]. The two nucleotide binding domains (NBD) domains show homology to the corresponding RecA domains of the DEAD helicase family [31]. This motor domain couples ATP binding and hydrolysis to conformational changes in other regions of the protein. In case of the monomeric PrcA helicase, alternating changes in the affinity of RecA domains for the substrate result in the translocation of the protein along the DNA by means of an *inchworm mechanism* [79,86]. Due to ATP binding, the two RecA domains move towards each other and swap their binding affinities for a single strand of the DNA. Subsequent ATP hydrolysis results in the separation of the two domains where the previous weakly binding domain now tightly holds on a single strand of DNA. This generated movement of the domains results in the unwinding of the double stranded DNA by PrcA helicase [79]. Because of the similarity of the DEAD motor domain, SecA has been proposed to function in a similar manner as helicases [55].

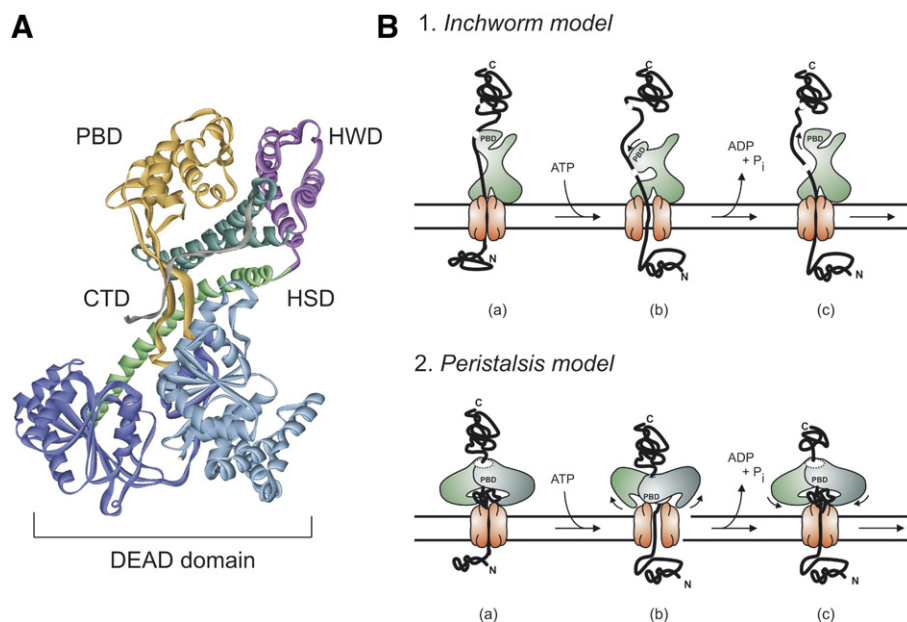


Fig. 2. SecA motor protein. (A) The structure of *B. subtilis* SecA protomer (1M6N PBD) [26] in which the individual domains are colored: blue, DEAD domain; yellow, protein binding domain (PBD); green, helical scaffold domain (HSD); violet, helical wing domain (HWD); and grey, C-terminal domain (CTD). (B) Schematic representation of SecA translocation mechanisms (1) *Inchworm model* – based on the open monomeric [55] and closed dimeric [26] structure of the *B. subtilis* SecA. (a) Nucleotide-free SecA (closed) bound at the PCC binds the preprotein via the PBD domain; (b) Binding of ATP results in conformational changes (arrow) at the PBD (open) which push the polypeptide into the PCC; (c) Upon ATP hydrolysis the polypeptide is released and the PBD reverts to the closed position. The retrograde movement of polypeptide is blocked by interactions with the PCC. The release of ADP stimulates SecA to bind the next segment of the preprotein. (2) *Peristalsis model* – based on the SecA dimer structures from *M. tuberculosis* (open pore) [66] and *B. subtilis* (closed pore) [26]. (a) The SecA dimer in an open pore conformation binds to the PCC (a dimer of SecYEG) creating a large central cavity in between PCC and SecA. Due to the Brownian motion, the polypeptide passes through the SecA pore into the cavity where the signal sequence binds to the PBD of one of the SecA protomers and the remainder of the cavity fills up with protein, possibly also involving the PBD of the other SecA protomer; (b) Conformational changes (arrow) due to ATP binding result in the closing of the SecA pore, the release of the preprotein from the PBD(s) and the opening of the PCC. The conformational change of the SecA dimer results in a reduction of the cavity volume, and the polypeptide is forced to move into the PCC channel; (c) ATP hydrolysis reverse the SecA conformational change, which results in the re-opening of the SecA pore and the closure of the PCC channel allowing a new stretch of preprotein to enter the SecA/PCC cavity. PCC – red, SecA – green. Arrows – direction of conformational changes in SecA.

SecA has been shown to function as a dimer [13,9,29]. Studies with SecA mutants that appear monomeric in solution suggest that a low level of activity may be associated with the monomer [54,53]. However, using a different type of biochemical analysis active variants we found to be dimeric [32]. In the crystal structures of the soluble protein, various types of dimer arrangements have been observed: i.e., an antiparallel – head to tail dimer [26,66,57,87] or a parallel – head to head dimer [78]. These dimers comprise similar SecA protomer structures. Structural and functional data indicate a communication between the DEAD and the preprotein binding domains (PBD) of individual SecA monomers (for review see [82]). In the monomeric nucleotide-free form of a *Bacillus subtilis* SecA mutant, a large conformational change of the PBD domain has been observed [55], and it was suggested that SecA may function according to the *inchworm mechanism* analogous to PrcA (Fig. 2B). However, DEAD helicases contain two substrate-binding sites with different affinities, and so far for the monomeric SecA only one peptide binding site has been detected. Therefore, the PCC was implicated as the second peptide site allowing SecA to rebind to the PCC trapped preprotein [54]. In the absence of SecA, preproteins can freely diffuse in the PCC [64,3] suggesting that such a binding site is not present.

A head-to-head type of dimer arrangement has been observed for the crystal form of SecA from *Thermus thermophilus* [78]. In this structure, the two monomers are positioned

parallel to each other, resembling “open scissors” with the DEAD motor at the bottom and the C-terminal domains (PBD and CTD) at the top creating a big opening between the monomers. It was speculated that the nucleotide-dependent conformational changes communicated via the DEAD domains to the PBD domains result in the opening or closing of the *scissors* with the preprotein attached to the top. Although the exact mechanism of translocation was not further specified, a Power stroke may occur through alternating interactions of the PBD domains of each monomer with the preprotein substrate.

In the crystal structures of dimeric *B. subtilis* [26] and *Mycobacterium tuberculosis* [66] SecA, an antiparallel head-to-tail organization of the monomers was observed. These structures, however, exhibit different dimer interfaces. The antiparallel SecA dimer shows a central opening, possibly a pore. While the *B. subtilis* SecA dimer seems to correspond to a relatively compact state with a narrow central opening [26], the *M. tuberculosis* SecA dimer has a more flat structure and a large central opening [66]. In the *piston model* [66], the preprotein is envisioned to be trapped in the central pore of the SecA dimer and is pushed through the PCC. This macromechanical movement would be generated by nucleotide-induced conformational changes of SecA as discussed before. This hypothesis requires that the central opening in the SecA dimer aligns with the pore of the PCC. This model was further refined in the

molecular peristalsis model (Fig. 2C) [48]. By analogy with the structural organization of the PCC associated with the ribosome, it was proposed that the active PCC consists of a 'front-to-front' SecYEG dimer. The SecA dimer may dock onto the SecYEG dimer by quasi-symmetrical interactions resulting in a large central cavity in between these two protein complexes. During the translocation process, the preprotein gains access to the PBDs that localize in this cavity by passing through the central pore of the SecA dimer. Upon ATP binding, the SecA pore closes, resulting in a more compact state of the SecA dimer, trapping of the preprotein and a concomitant decrease in the cavity volume. The simultaneous opening of the central pore in the PCC would be brought about by a reduction of the distance between the interactions sites of the individual SecA monomers with the cytosolic loop regions of the two SecY proteins (Box 1). In this manner, the reduction of the cavity size is directly coupled to opening of the PCC allowing the directed diffusion of the cavity entrapped preprotein segment across the membrane, while the remainder of the preprotein would stay trapped in the central pore of the SecA dimer. Notably, in this model SecA functions through a mechanism that combines the main working models as the actual movement of the polypeptide through the pore occurs by Brownian motion while a Power stroke is employed to decrease the cavity size and to open the PCC. A free diffusional mechanism of translocation is also supported by observations that in the absence of SecA association, protein translocation intermediates can undergo reverse movements within the translocation channel [64,3].

Although the *step-size* mechanism of translocation has so far only been demonstrated for only one single model protein [64,77], it is of interest to relate the translocation progress of about 20 amino acids per sub-step to the proposed working mechanisms of SecA. In the *inchworm* or *piston* mechanisms, the step size will depend on the size of the lever arm. Can such a conformational change reach a distance of ~ 66 Å (1 amino acid ~ 3.3 Å)? Interestingly, the large step size might be explained by the *peristalsis* mechanism, as this will be determined only by the volume of the proposed cavity [48].

5. Trans-acting motor proteins – similar molecular motors, different channels

In contrast to *cis*-acting motors like SecA, motor proteins can also be localized on the *trans* side of the membrane. This is exemplified by BiP and Hsp70, which drive protein import into the ER and mitochondria, respectively. Even though the translocation channels differ significantly between these two systems, the molecular motors show a remarkable similarity, i.e. they both belong to the family of Hsp70 chaperones (reviewed in [45]). Hsp70 chaperones comprise an N-terminal ATPase domain (NBD) and a C-terminal peptide binding domain (PBD). The current model of the catalytic cycle of Hsp70-like proteins (hereafter Hsp70) is based on the cytosolic chaperone DnaK of *E. coli* (Fig. 3) [45]. Generally, the nucleotide occupancy of the NBD controls the peptide-binding affinity of the PBD. In the ATP-bound state, DnaK shows a low affinity for peptides, whereas polypeptides are tightly bound in the ADP-bound state. Interconversion between both states is regulated by DnaJ, a specific J-domain protein (JDP) that stimulates the hydrolysis of ATP by DnaK, and by GrpE,

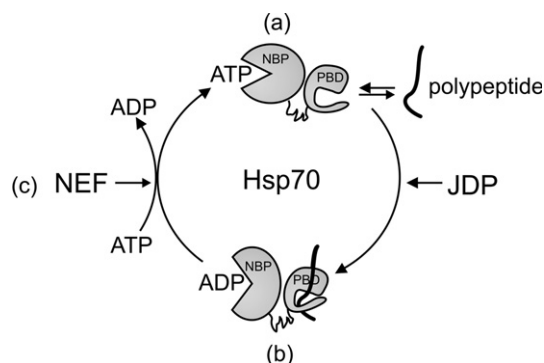


Fig. 3. Model of the Hsp70 chaperone cycle (adopted from [45]). (a) Hsp70 (DnaK) in the ATP-bound state interacts with the polypeptide via its PBD in an open conformation (low affinity). (b) ATP hydrolysis stimulated by a JDP (DnaJ) and the polypeptide substrate closes the PBD (high affinity). (c) Binding of the NEF (GrpE) catalyzes the release of ADP. Subsequently, binding of ATP opens the PBD (low affinity) and releases the polypeptide.

a nucleotide exchange factor (NEF) that facilitates ADP-release.

Based on the working model for DnaK, two different mechanisms for Hsp70-driven protein transport have been proposed: the Brownian ratchet and Power stroke models (Fig. 1B) [22,59]. In both models multiple cycles of nucleotide-dependent trapping or pulling of the preprotein by Hsp70 effectuate translocation. Additionally, efficient binding of the incoming polypeptide requires membrane anchoring of the Hsp70 protein. In the Power stroke model, the membrane anchor also serves as a molecular fulcrum. One of the main differences between both models relates to the amount of force exerted by Hsp70 on the polypeptide. In the Brownian ratchet model, the preprotein domains unfold and refold spontaneously at a millisecond scale ("thermal breathing"), and the forces exerted by Hsp70 are relatively small. Hsp70 would merely provide directionality to the folding process through processive trapping of protein unfolding states. In the power-stroke model, Hsp70 accelerates translocation by increasing the unfolding rate through active pulling which requires larger forces. Therefore, the expected rate of translocation will be constant and faster than predicted for the Brownian ratchet mechanism. The next section discusses the proposed protein import mechanisms into the ER and mitochondria.

6. Trans-acting motor protein – BiP

Most proteins are translocated co-translationally across the ER membrane, but post-translational import via the Sec61p complex (See Box 1) also occurs and involves the *trans*-acting motor protein BiP (also referred to as luminal Hsp70) in conjunction with the membrane protein Sec63 [6,42]. Initially, BiP was suggested to pull on the incoming polypeptide [22]. However, further studies demonstrated that translocation can be driven solely by trapping the translocated protein by a luminal antibody, and apparently does not require multiple cycles of BiP activity [42]. This suggests that BiP may rather act as a molecular ratchet that, possibly by a single ATP hydrolysis cycle, traps the substrate and directs its translocation by preventing retrograde movement [42]. However, antibody-dependent

translocation is less efficient than BiP mediated translocation, suggesting a more complex mechanism than trapping only. Sec63 is essential for post-translational translocation and serves as a membrane-anchor for BiP while regulating the hydrolysis of ATP via its J-domain [47]. Binding of BiP to Sec63 increases the substrate binding affinity and places BiP in the most effective trapping position, close to the exit channel [47]. Next, BiP may actively pull on the polypeptide in addition to the trapping. However, the interaction between BiP and Sec63 is short lived (transient), while for an active pulling mechanism a more stable interaction would be expected [46]. Sec63 possesses a large cytoplasmic domain needed for assembly of the translocase [28]. Together with other translocase associated proteins, i.e. Sec62, Sec71 and Sec72, the cytoplasmic domain of Sec63 may bind the preprotein at an early stage of the translocation reaction [41]. Consequently, Sec63 may fulfill an additional regulatory function by sensing preprotein binding at the *cis*-side and transferring this information to the motor protein (BiP) that would be primed to accept preproteins at the *trans*-side of the membrane.

Mathematical modeling based on the biochemical experiments has been employed to shed light on the mechanism of post-translational translocation into the ER. These analyses suggest that a Brownian ratchet as well as a Power stroke can explain this process [17,37,18]. Proteins imported into the ER frequently undergo a process of maturation [30,72]. It was shown that Sec61p interacts with maturation complexes such as the signal peptidase or oligosaccharyl transferase [85,7]. The removal of the signal sequence as well as *N*-glycosylation seems to occur before protein folding and may affect subsequent folding pathways. Additionally, formation of disulfide-bonds may influence the stability of protein folding. It seems conceivable that each of these processes could serve as an additional trapping element that regulates the rate and direction of translocation.

Interestingly, the function of BiP is not limited to post-translational translocation, as it also increases the fidelity of co-translational translocation [6]. Moreover, BiP in its ADP-bound state has been proposed to act as a gatekeeper to prevent ion leakage through the PCC by sealing the pore from the lumen site [23,1]. This seal is released upon the binding of ATP to BiP. A physical interaction of BiP with Sec61p remains to be demonstrated. BiP may indirectly affect the pore conformation via another yet unknown translocation-associated protein [1]. Also additional J-domain proteins (ERdj1-4) [5,71,68,67] may have distinct roles in ER import.

7. Trans-acting motor protein – mtHsp70

Protein import into mitochondria is dependent on the mtHsp70 protein that is part of the import motor (presequence translocase-associated motor, PAM). The import motor further comprises Tim44, the JDP Pam18 and the NEF Mge1 [51]. The import motor acts at the *trans*-side of the inner mitochondrial membrane. Before gaining access to the import motor, preproteins need to pass the outer and inner membrane via the TOM40 and TIM23/17 complexes, respectively (Fig. 1). The initiation of translocation is driven by the $\Delta\psi$, likely by an electrophoretic effect on the signal sequence [60]. Additionally, $\Delta\psi$ supports PAM driven-translocation by stabilizing the polypeptide in the channel, thereby increasing the efficiency of the interaction

between the preprotein and mtHsp70 on the *trans*-side [33]. Mitochondrial protein import occurs mostly post-translational and in order to pass both membranes through the narrow channels, preproteins need to be largely unfolded. In the working model for the preprotein import into the matrix, the dimer of the Tim23/17 complex cooperates with two import motors. Here the forward preprotein movement is promoted by the cooperative sequential binding of two mtHsp70 proteins to an incoming polypeptide in a “hand over hand” manner [50].

How does the import motor drive the translocation reaction? Again two models have been proposed; the Brownian ratchet [20,52,40] and Power stroke [43,44] mechanism (Fig. 1B). Several lines of evidence point at a Brownian ratchet mechanism of protein import. Upon depletion of ATP, which results in a loss of interaction between mtHsp70 and the translocating polypeptide, retrograde movements of intermediates have been observed [52]. Furthermore, introduction of poly-glycine or poly-glutamate stretches in the polypeptide chain, to disfavor binding of mtHsp70, did not affect the translocation [52]. Together, these results indicate that in the absence of an active mtHsp70, a spontaneous translocation progress of the unfolded preprotein is possible once translocation is initiated by the $\Delta\psi$ [52]. In addition to the JDP Pam18, mtHsp70 also interacts with the membrane protein Tim44. Tim44 recruits mtHsp70 near to the Tim23/17 channel exit [50]. In a mtHsp70 mutant (*sscl-2*) that is affected in Tim44 binding, import of tightly folded proteins is abolished, whereas unfolded proteins are translocated already at low $\Delta\psi$ [81,80]. Furthermore, loosely folded polypeptides are translocated more efficiently by the *sscl-2* mutant than by wild type mtHsp70 [21]. These observations have led to the suggestion that different mechanisms for mitochondrial import may exist depending on the characteristics of the preprotein. Translocation of tightly folded domains may require a larger force, while trapping could be a predominant mechanism for loosely folded polypeptide chains [80,21]. It remains to be established whether the interaction of mtHsp70 with Tim44 or Pam18 indeed serves as a fulcrum to provide a force sufficiently large to translocate the preprotein by active pulling, or whether this merely serves to increase the efficiency of trapping by raising the concentration of mtHsp70 at the channel exit.

The protein structure, i.e., the length of the signal peptide and the local structure of the N-terminal region of the mature domain dramatically affect the mitochondrial translocation rate [43,38,24,84]. Studies on protein folding suggest that the translocase influences the unfolding pathway by unraveling the preprotein from its N-terminus either by an active or passive mechanism, resulting in the collapse of the protein structure [43,25,63,73]. Since the initiation of local unfolding will result in the cooperative, full unfolding of the protein, the maximum translocation rate would not depend on the initiation phase but on the intrinsic property of the import motor to move unfolded polypeptides through the PCC [38]. Recently a new model, *entropic pulling*, was proposed for luminal Hsp70 driven translocation [10], in which the binding of Hsp70 to the emerging polypeptide decreases the freedom of Brownian movements due to the large volume of Hsp70. Upon release of peptide-bound mtHsp70 from Tim44, an entropic pulling force is generated. By this mechanism Hsp70 entropically pulls (in the absence of a molecular fulcrum) on the polypeptide which is translocated by a Brownian motion. Entropic pulling thus effectively connects the power stroke and Brown-

ian ratchet mechanisms which both require mtHsp70 and Tim44 for efficient translocation.

8. Concluding remarks

All protein translocation systems described above consist of at least two parts: a membrane channel and an associated motor protein. Intriguingly, motor proteins act at different sides of the membrane, and this mostly likely relates to the availability of the energy source. Though ATP is present in both the lumen of the ER and the matrix of the mitochondria, no ATP is available in the bacterial periplasm. The presence of *trans*-acting motors in the lumen of the ER and the mitochondrial matrix ensure a high local concentration of such motor proteins at the translocation sites, which would be more difficult to achieve at the *cis*-position in the cytosol. Curiously, post-translational translocation across the cytoplasmic membrane has also been observed in Archaea [27] even though these prokaryotes lack a SecA homolog to complement their highly conserved PCC. Therefore, it is an unsolved question how post-translational protein translocation is mediated in Archaea.

Folding at the *cis*-side opposes translocation. Can refolding on the *trans*-side stimulate the translocation process? In some bacteria, SecB is a cytosolic translocation-dedicated chaperone that maintains the preprotein in a loosely folded state [34,39] and transfers it to SecA. SecA possibly further stabilizes the translocation competent state of the preprotein [15] consistent with a proposed chaperone activity of SecA [19]. Moreover, preproteins can move passively through the PCC in the absence of SecA association [64,12,3]. For SecB-dependent protein translocation only little energy may be spent on active unfolding events while most of the energy would be used to direct the movement of polypeptide. *Cis*-acting motors, like SecA seem to prevent the retrograde movement of the polypeptide allowing them to fold on the *trans*-side, whereas *trans*-acting motors, like BiP and mtHsp70, additionally may accelerate folding of the translocated protein, consistent with a role of these chaperones in protein folding [70]. Importantly, alternative functions for the motor proteins during the translocation reaction should be considered, such as controlling the opening and closure of the translocation pore. This function has been proposed for SecA [48] and mtHsp70 [11,58]. In this respect, BiP has also been implicated in controlling the opening and closure of the PCC at the luminal side of the ER.

The experimental validation of a Brownian motion (trapping) and power stroke (pulling/pushing) remains very difficult and possibly a hybrid mechanism concurs. Ultimately, further structural data on the molecular motors in complex with the PCC and a preprotein will deepen our insight in the molecular mechanism of motor action.

Acknowledgements: This work was supported by the Foundation for Fundamental Research on Matter (FOM), Earth and Life Sciences (ALW), the Royal Academy of Sciences of the Netherlands (KNAW), and NanoNed, a national nanotechnology program coordinated by the Dutch Ministry of Economic Affairs.

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